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(54) Title: IMPROVEMENTS IN VACCINATION

(57) Abstract: The present invention relates to improvements in DNA vaccination and in particular, methods of vaccinating a mammal against disease states, and to the use of imidazo [4,5 -c] quinolin - 4 - amine derivative adjuvants in the manufacture of medicaments for boosting previously vaccinated individuals.

Improvements in Vaccination

5 The present invention relates to improvements in DNA vaccination and in particular, methods of vaccinating a mammal against disease states, and to the use of certain compounds in the manufacture of medicaments for boosting previously vaccinated individuals.

10 Traditional vaccination techniques which involve the introduction into an animal system of an antigen which can induce an immune response in the animal, and thereby protect the animal against infection, have been known for many years. Following the observation in the early 1990's that plasmid DNA could directly transfect animal cells *in vivo*, significant research efforts have been undertaken to develop vaccination techniques based upon the use of DNA plasmids to induce immune responses, by direct introduction into animals of DNA which encodes for antigenic peptides. Such techniques, which are referred to as "DNA immunisation" or "DNA vaccination" have now been used to elicit 15 protective antibody (humoral) and cell-mediated (cellular) immune responses in a wide variety of pre-clinical models for viral, bacterial and parasitic diseases. Research is also underway in relation to the use of DNA vaccination techniques in treatment and protection against cancer, allergies and autoimmune diseases.

20 DNA vaccines usually consist of a bacterial plasmid vector into which is inserted a strong promoter, the gene of interest which encodes for an antigenic peptide and a polyadenylation/transcriptional termination sequence. The immunogen which the gene of interest encodes may be a full protein or simply an antigenic peptide sequence relating to the pathogen, tumour or other agent which is intended to be protected against. The plasmid can be grown in bacteria, such as for example *E. coli* and then isolated and 25 prepared in an appropriate medium, depending upon the intended route of administration, before being administered to the host.

Helpful background information in relation to DNA vaccination is provided in "Donnelly, J *et al Annual Rev. Immunol.* (1997) 15:617-648, the disclosure of which is included herein in its entirety by way of reference.

30 There are a number of advantages of DNA vaccination relative to traditional vaccination techniques. First, it is predicted that because the proteins which are encoded by the DNA sequence are synthesised in the host, the structure or conformation of the

protein will be similar to the native protein associated with the disease state. It is also likely that DNA vaccination will offer protection against different strains of a virus, by generating cytotoxic T lymphocyte responses that recognise epitopes from conserved proteins. Furthermore, because the plasmids are introduced directly to host cells where antigenic protein can be produced, a long-lasting immune response will be elicited. The technology also offers the possibility of combining diverse immunogens into a single preparation to facilitate simultaneous immunisation in relation to a number of disease states.

Despite the numerous advantages associated with DNA vaccination relative to traditional vaccination therapies, there is nonetheless a desire to develop adjuvant compounds which will serve to increase the immune response induced by the protein which is encoded by the plasmid DNA administered to an animal.

DNA vaccination is sometimes associated with an inappropriate deviation of immune response from a Th1 to a Th2 response, especially when the DNA is administered directly to the epidermis (Fuller and Haynes *Hum. Retrovir.* (1994) 10:1433-41). It is recognised that the immune profile desired from a nucleic acid vaccine depends on the disease being targeted. The preferential stimulation of a Th1 response is likely to provide efficacy of vaccines for many viral diseases and cancers, and a dominant Th2 type of response may be effective in limiting allergy and inflammation associated with some autoimmune diseases. Accordingly, ways to quantitatively raise the immune response or to shift the type of response to that which would be most efficacious for the disease indication, may be useful.

Accordingly, it is one object of the present invention to provide novel vaccination protocols which can be used in conjunction with DNA vaccination procedures.

Imidazoquinolineamine derivatives are inducers of cytokines, including IFN- α , IL-6 and TNF- α (See, e.g. Reiter et al, *J. Leukocyte Biology* (1994) 55:234-240). These compounds and processes for their preparation have been disclosed in PCT patent application publication number WO 94/17043.

The use of Imidazoquinolineamine derivatives as adjuvants is disclosed WO 02/24225. This document discloses the fact that such adjuvants may be used at both priming and booster doses of DNA vaccines. There is no disclosure that the immune responses may be further enhanced by the methods of the present invention.

The present inventors have shown, surprisingly, that it is advantageous for imidazo [4,5-c] quinolin-4-amine derivative adjuvants to be used in boosting DNA vaccines, and used to boost immune responses initiated using DNA priming vaccines that do not comprise the imidazo [4,5-c] quinolin-4-amine derivatives.

5 In the preferred methods of the present invention the antigen is a nucleic acid encoding a protein against which it is desired to create an immune response.

The present invention provides a method of vaccinating an individual comprising the steps of:

(a) vaccinating the individual with a first vaccine on one or more occasions, 10 characterised in that said vaccine composition comprises an antigen but does not comprise an imidazo [4,5-c] quinolin-4-amine derivative, and

(b) after waiting an appropriate length of time, vaccinating the same individual one of more times with a second vaccine, characterised in that the second vaccine composition comprises the same antigen and is administered with an imidazo [4,5-c] quinolin-4-amine derivative.

In an embodiment of the present invention, the method of vaccinating an individual further comprises a repeat of step (a) after step (b). In an alternate embodiment of the present invention the method of vaccinating an individual comprises two administrations of the first vaccine composition in step (a).

20 The administration of the second vaccine composition may comprise the simultaneous or sequential administration of the imidazo [4,5-c] quinolin-4-amine derivative and the antigen. Also envisaged are methods wherein the second vaccine composition comprises the imidazo [4,5-c] quinolin-4-amine derivative and the antigen administered different sites.

25 In the context of the present invention, the "antigen" present in the second vaccine is a polynucleotide that encodes a polypeptide against which an immune response is desired to be raised. Preferably the antigen is a polynucleotide in both the first and second vaccines.

30 Also provided by the present invention is a method of increasing the frequency of antigen specific Interferon- γ (IFN- γ) producing cells in an individual comprising

(a) administering to that individual a first vaccine composition on one or more occasions, characterised in that said first vaccine composition comprises an antigen but does not comprise an imidazo [4,5-c] quinolin – 4 – amine derivative,

(b) after waiting an appropriate length of time, administering to the same individual a second vaccine composition on one or more occasions, characterised in that the second vaccine composition comprises the same antigen and an imidazo [4,5-c] quinolin – 4 – amine derivative.

In an embodiment of the present invention, the method increasing the frequency of antigen specific Interferon- γ (IFN- γ) producing cells further comprises a repeat of step (a) after step (b).

It is preferred in the methods described above, that the second or “booster” vaccine comprising the imidazo [4,5-c] quinolin – 4 – amine derivative is the final vaccine dose administered. That is to say that the vaccinee may receive one or more doses of the vaccine (without the imidazo [4,5-c] quinolin – 4 – amine derivative) followed by a final boosting dose of the second vaccine composition (with the imidazo [4,5-c] quinolin – 4 – amine derivative).

The present invention also provides the use of an imidazo [4,5-c] quinolin – 4 – amine derivative and an antigen in the manufacture of a booster dose of a vaccine medicament for administration to an individual, characterised in that the individual previously received one or more priming doses of the vaccine medicament comprising the same antigen but which did not comprise an imidazo [4,5-c] quinolin – 4 – amine derivative.

In a related aspect of the present invention is a vaccine administration device comprising an antigen and an imidazo [4,5-c] quinolin – 4 – amine derivative, the device being packaged together with an instruction leaflet advising that the administration device is used to administer the vaccine composition only to individuals that had previously received a vaccine comprising the same antigen but which did not comprise an imidazo [4,5-c] quinolin – 4 – amine derivative.

There is also provided a kit comprising a first vaccine composition and a second vaccine composition, wherein the first vaccine composition and the second composition contain the same antigen characterised in that the second vaccine composition comprises an imidazo [4,5-c] quinolin – 4 – amine derivative.

Preferably the 1H-imidazo[4,5-c]quinolin-4-amine-derivative is a compound defined by one of formulae I-VI defined herein. More preferably, it is a compound defined by formula VI. Particularly preferred is when the 1H-imidazo[4,5-c]quinolin-4-amine derivative is a compound of formula VI selected from the group consisting of :

5 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine;
1-(2-hydroxy-2-methylpropyl)-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine;
1-(2,hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine;
1-(2-hydroxy-2-methylpropyl)-2-ethoxymethyl-1-H-imidazo[4,5-c]quinolin-4-amine. The most preferred derivative is imiquimod.

10 Throughout this specification and the appended claims, unless the context requires otherwise, the words "comprise" and "include" or variations such as "comprising", "comprises", "including", "includes", etc., are to be construed inclusively, that is, use of these words will imply the possible inclusion of integers or elements not specifically recited.

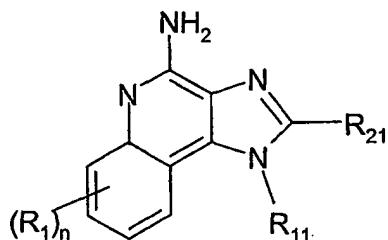
15 As described above, the present invention relates vaccination methods, and to improvements of methods of vaccination involving the introduction into a mammal of nucleotide sequence which encodes for an immunogen which is an antigenic protein or peptide, such that the protein or peptide will be expressed within the mammalian body to thereby induce an immune response within the mammal against the antigenic protein or peptide. Such methods of vaccination are well known and are fully described in 20 Donnelly *et al* as referred to above.

25 As used herein the term vaccine composition, in the context of the second or booster vaccine composition, refers to a combination of a immunogen component comprising a nucleotide sequence encoding an immunogen, and an adjuvant component comprising a 1H-imidazo [4,5-c] quinolin-4-amine derivative. The combination is, for example, in the form of an admixture of the two components in a single pharmaceutically acceptable formulation or in the form of separate, individual components, for example in the form of a kit comprising an immunogen component comprising the nucleotide sequence encoding an immunogen, and an adjuvant component comprising the 1H-imidazo[4,5-c]quinolin-4-amine, wherein the two components are for separate, sequential or simultaneous 30 administration. Preferably, the administration of the two components is substantially simultaneous.

The 1H-imidazo[4,5-c]quinolin-4-amine derivative as referred to throughout the specification and the claims is preferably a compound defined by one of Formulas I-VI below:

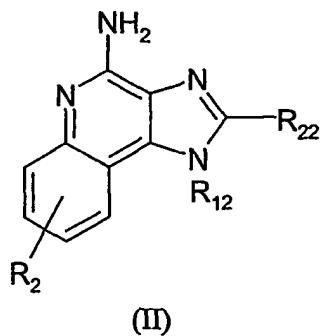
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(I)



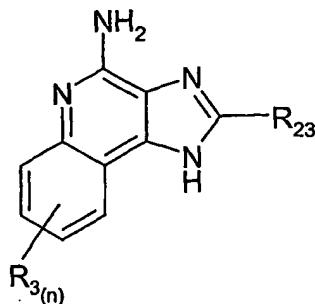
wherein

R₁₁ is selected from the group consisting of straight or branched chain alkyl,
 10 hydroxyalkyl, acyloxyalkyl, benzyl, (phenyl)ethyl and phenyl, said benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms and halogen, with the proviso that if said benzene ring is substituted by two of said moieties, then said moieties together contain no more than 6 carbon atoms; R₂₁ is selected from the group consisting of hydrogen, alkyl of one to about eight carbon atoms, benzyl, (phenyl)ethyl and phenyl, the benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms and halogen,
 15 with the proviso that when the benzene ring is substituted by two of said moieties, then the moieties together contain no more than 6 carbon atoms; and each R₁ is independently selected from the group consisting of hydrogen, alkoxy of one to about four carbon atoms, halogen and alkyl of one to about four carbon atoms, and n is an integer from 0 to 2, with the proviso that if n is 2, then said R₁₁ groups together contain no more than 6
 20 carbon atoms;
 25



wherein

R₁₂ is selected from the group consisting of straight chain or branched chain alkenyl containing 2 to about 10 carbon atoms and substituted straight chain or branched chain alkenyl containing 2 to about 10 carbon atoms, wherein the substituent is selected from the group consisting of straight chain or branched chain alkyl containing 1 to about 4 carbon atoms and cycloalkyl containing 3 to about 6 carbon atoms; and cycloalkyl containing 3 to about 6 carbon atoms substituted by straight chain or branched chain alkyl containing 1 to about 4 carbon atoms; and R₂₂ is selected from the group consisting of hydrogen, straight chain or branched chain alkyl containing one to about eight carbon atoms, benzyl, (phenyl)ethyl and phenyl, the benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of straight chain or branched chain alkyl containing one to about four carbon atoms, straight chain or branched chain alkoxy containing one to about four carbon atoms, and halogen, with the proviso that when the benzene ring is substituted by two such moieties, then the moieties together contain no more than 6 carbon atoms; and each R₂ is independently selected from the group consisting of straight chain or branched chain alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms, and n is an integer from zero to 2, with the proviso that if n is 2, then said R₂ groups together contain no more than 6 carbon atoms;



(III)

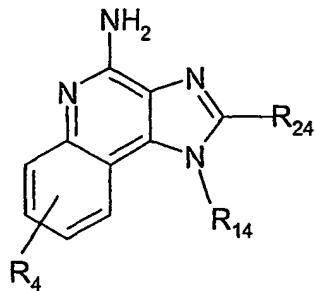
5

wherein

R₂₃ is selected from the group consisting of hydrogen, straight chain or branched chain alkyl of one to about eight carbon atoms, benzyl, (phenyl)ethyl and phenyl, the benzyl,

10 (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of straight chain or branched chain alkyl of one to about four carbon atoms, straight chain or branched chain alkoxy of one to about four carbon atoms, and halogen, with the proviso that when the benzene ring is substituted by two such moieties, then the moieties together- contain no

15 more than 6 carbon atoms; and each R₅ is independently selected from the group consisting of straight chain or branched chain alkoxy of one to about four-carbon atoms, halogen, and 30 straight chain or branched chain alkyl of one to about four carbon atoms, and n is an integer from zero to 2, with the proviso that if n is 2, then said R₃ groups together contain no more than 6 carbon atoms;



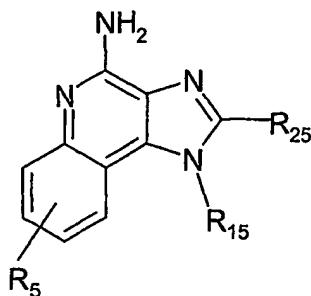
(IV)

wherein

5 R_{14} is $-\text{CH}_2\text{R}_A\text{R}_B$ wherein R_B is hydrogen or a carbon-carbon bond, with the proviso that when R_B is hydrogen R_A is alkoxy of one to about four carbon atoms, hydroxyalkoxy of one to about four carbon atoms, 1-alkynyl of two to about ten carbon atoms, tetrahydropyranyl, alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about four carbon atoms, 2-, 3-, or 4-pyridyl, and with the further proviso that when R_B is a carbon-carbon bond R_B and R_A together form a tetrahydrofuryl group optionally substituted with one or more substituents independently selected from the group consisting of hydroxy and hydroxyalkyl of one to about four carbon atoms; R_{24} is selected from the group consisting of hydrogen, alkyl of one to about four carbon atoms, phenyl, and substituted phenyl

10 wherein the substituent is selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms, and halogen; and R_4 is selected from the group consisting of hydrogen, straight chain or branched chain alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms;

15 .



(V)

wherein

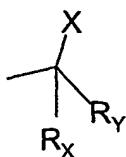
5 R_{15} is selected from the group consisting of: hydrogen; straight chain or branched chain alkyl containing one to about ten carbon atoms and substituted straight chain or branched chain alkyl containing one to about ten carbon atoms, wherein the substituent is selected from the group consisting of cycloalkyl containing three to about six carbon atoms and cycloalkyl containing three to about six carbon atoms substituted by straight chain or

10 branched chain alkyl containing one to about four carbon atoms; straight chain or branched chain alkenyl containing two to about ten carbon atoms and substituted straight chain or branched chain alkenyl containing two to about ten carbon atoms, wherein the substituent is selected from the group consisting of cycloalkyl containing three to about six carbon atoms and cycloalkyl containing three to about six carbon atoms substituted by

15 straight chain or branched chain alkyl containing one to about four carbon atoms; hydroxyalkyl of one to about six carbon atoms; alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about six carbon atoms; acyloxyalkyl wherein the acyloxy moiety is alkanoyloxy of two to about four carbon atoms or benzyloxy, and the alkyl moiety contains one to about six carbon atoms; benzyl; (phenyl)ethyl; and phenyl; said benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms, and halogen, with the proviso that when said benzene ring is substituted by two of said moieties, then the moieties together contain no more

20 than six carbon atoms;

25 R_{25} is



wherein

R_X and R_Y are independently selected from the group consisting of hydrogen, alkyl of one to about four carbon atoms, phenyl, and substituted phenyl wherein the substituent is selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms, and halogen; X is selected from the group consisting of alkoxy containing one to about four carbon atoms, alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about four carbon atoms, haloalkyl of one to about four carbon atoms, alkylamido wherein the alkyl group contains one to about four carbon atoms, amino, substituted amino wherein the substituent is alkyl or hydroxyalkyl of one to about four carbon atoms, azido, alkylthio of one to about four carbon atoms; and R₅ is selected from the group consisting of hydrogen, straight chain or branched chain alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms; or a pharmaceutically acceptable salt of any of the foregoing.

Preferred alkyl groups are C₁ – C₄ alkyl, for example methyl, ethyl, propyl, 2-methylpropyl and butyl. Most preferred alkyl groups are methyl, ethyl and 2methylpropyl. Preferred alkoxy groups are methoxy, ethoxy and ethoxymethyl.

The compounds recited above and methods for their preparation are disclosed in PCT patent application publication number WO 94/17043.

In instances where n can be zero, one, or two, n is preferably zero or one.

The substituents R₁-R₅ above are generally designated "benzo substituents" herein.

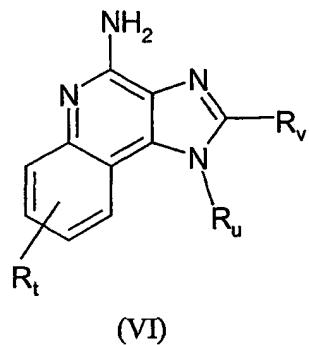
The preferred benzo substituent is hydrogen.

The substituents R₁₁-R₁₅ above are generally designated "1-substituents" herein. The preferred 1-substituent is 2-methylpropyl or 2-hydroxy-2-methylpropyl.

The substituents R₂₁-R₂₅ above are generally designated "2-substituents", herein. The preferred 2-substituents are hydrogen, alkyl of one to about six carbon atoms, alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about four carbon atoms. Most preferably the 2-substituent is hydrogen, 5 methyl, or ethoxymethyl.

Particularly preferred is when the 1H-imidazo[4,5-c]quinolin-4-amine is a compound defined by formula VI below:

10



15

Wherein

R₁ is selected from the group consisting of hydrogen, straight chain or branched chain alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms;

20 R₂ is 2-methylpropyl or 2-hydroxy-2-methylpropyl; and

R₃ is hydrogen, alkyl of one to about six carbon atoms, or alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about four carbon atoms; or physiologically acceptable salts of any of the foregoing, where appropriate.

In formula VI, R_t is preferably hydrogen, R_u is preferably 2-methylpropyl or 2-hydroxy-2-methylpropyl, and R_v is preferably hydrogen, methyl or ethoxymethyl.

Preferred 1H-imidazo[4,5-c]quinolin-4-amines include the following:

1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine (a compound of formula VI
5 wherein R_t is hydrogen, R_u is 2-methylpropyl and R_v is hydrogen);

1-(2-hydroxy-2-methylpropyl)-2-methyl-1H-imidazo[4,5-c]quinolin-4-amine (a compound of formula VI wherein R_t is hydrogen, R_u is 2-hydroxy-2-methylpropyl, and R_v is methyl);

10 1-(2-hydroxy-2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine (a compound of formula VI wherein R_t is hydrogen, R_u is 2-hydroxy-2-methylpropyl, and R_v is hydrogen)

15 1-(2-hydroxy-2-methylpropyl)-2-ethoxymethyl-1H-imidazo[4,5-c]quinolin-4-amine (a compound of formula VI wherein R_t is hydrogen, R_u is 2-hydroxy-2-methylpropyl and R_v is ethoxymethyl);

or physiologically acceptable salts thereof.

15 It is possible for the vaccination methods and compositions according to the present application to be adapted for protection or treatment of mammals against a variety of disease states such as, for example, viral, bacterial or parasitic infections, cancer, allergies and autoimmune disorders.

20 The nucleotide sequences referred to in this application, which are to be expressed within a mammalian system, in order to induce an antigenic response, may encode for an entire protein, or merely a shorter peptide sequence which is capable of initiating an antigenic response. Throughout this specification and the appended claims, the phrase "antigenic peptide" or "immunogen" is intended to encompass all peptide or protein sequences which are capable of inducing an immune response within the animal

25 concerned. Most preferably, however, the nucleotide sequence will encode for a full protein which is associated with the disease state, as the expression of full proteins within the animal system are more likely to mimic natural antigen presentation, and thereby evoke a full immune response.

30 Antigens which are capable of eliciting an immune response against a human pathogen, which antigen or antigenic composition is derived from HIV-1, (such as tat, nef, gp120 or gp160, gp40, p24, gag, env, vif, vpr, vpu, rev), human herpes viruses, such as gH, gL gM gB gC gK gE or gD or derivatives thereof or Immediate Early protein such

as ICP27, ICP 47, IC P 4, ICP36 from HSV1 or HSV2, cytomegalovirus, especially Human, (such as gB or derivatives thereof), Epstein Barr virus (such as gp350 or derivatives thereof), Varicella Zoster Virus (such as gpI, II, III and IE63), or from a hepatitis virus such as hepatitis B virus (for example Hepatitis B Surface antigen or Hepatitis core antigen or pol), hepatitis C virus antigen and hepatitis E virus antigen, or from other viral pathogens, such as paramyxoviruses: Respiratory Syncytial virus (such as F and G proteins or derivatives thereof), or antigens from parainfluenza virus, measles virus, mumps virus, human papilloma viruses (for example HPV6, 11, 16, 18, eg L1, L2, E1, E2, E3, E4, E5, E6, E7), flaviviruses (e.g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or Influenza virus cells, such as HA, NP, NA, or M proteins, or combinations thereof), or antigens derived from bacterial pathogens such as *Neisseria spp*, including *N. gonorrhoea* and *N. meningitidis*, eg, transferrin-binding proteins, lactoferrin binding proteins, PilC, adhesins); *S. pyogenes* (for example M proteins or fragments thereof, C5A protease, *S. agalactiae*, *S. mutans*; *H. ducreyi*; *Moraxella spp*, including *M catarrhalis*, also known as *Branhamella catarrhalis* (for example high and low molecular weight adhesins and invasins); *Bordetella spp*, including *B. pertussis* (for example pertactin, pertussis toxin or derivatives thereof, filamentous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis* and *B. bronchiseptica*; *Mycobacterium spp*, including *M. tuberculosis* (for example ESAT6, Antigen 85A, -B or -C, MPT 44, MPT59, MPT45, HSP10, HSP65, HSP70, HSP 75, HSP90, PPD 19kDa [Rv3763], PPD 38kDa [Rv0934]), *M. bovis*, *M. leprae*, *M. avium*, *M. paratuberculosis*, *M. smegmatis*; *Legionella spp*, including *L. pneumophila*; *Escherichia spp*, including enterotoxic *E. coli* (for example colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof), enterohemorrhagic *E. coli*, enteropathogenic *E. coli* (for example shiga toxin-like toxin or derivatives thereof); *Vibrio spp*, including *V. cholera* (for example cholera toxin or derivatives thereof); *Shigella spp*, including *S. sonnei*, *S. dysenteriae*, *S. flexnerii*; *Yersinia spp*, including *Y. enterocolitica* (for example a Yop protein), *Y. pestis*, *Y. pseudotuberculosis*; *Campylobacter spp*, including *C. jejuni* (for example toxins, adhesins and invasins) and *C. coli*; *Salmonella spp*, including *S. typhi*, *S. paratyphi*, *S. choleraesuis*, *S. enteritidis*; *Listeria spp*, including *L. monocytogenes*; *Helicobacter spp*, including *H. pylori* (for example urease, catalase, vacuolating toxin); *Pseudomonas spp*,

including *P. aeruginosa*; *Staphylococcus spp.*, including *S. aureus*, *S. epidermidis*; *Enterococcus spp.*, including *E. faecalis*, *E. faecium*; *Clostridium spp.*, including *C. tetani* (for example tetanus toxin and derivative thereof), *C. botulinum* (for example botulinum toxin and derivative thereof), *C. difficile* (for example clostridium toxins A or B and derivatives thereof); *Bacillus spp.*, including *B. anthracis* (for example botulinum toxin and derivatives thereof); *Corynebacterium spp.*, including *C. diphtheriae* (for example diphtheria toxin and derivatives thereof); *Borrelia spp.*, including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (for example OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, OspC, DbpA, DbpB), *B. andersonii* (for example OspA, OspC, DbpA, DbpB), *B. hermsii*; *Ehrlichia spp.*, including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis; *Rickettsia spp.*, including *R. rickettsii*; *Chlamydia spp.*, including *C. trachomatis* (for example MOMP, heparin-binding proteins), *C. pneumoniae* (for example MOMP, heparin-binding proteins), *C. psittaci*; *Leptospira spp.*, including *L. interrogans*; *Treponema spp.*, including *T. pallidum* (for example the rare outer membrane proteins), *T. denticola*, *T. hyoysenteriae*; or derived from parasites such as *Plasmodium spp.*, including *P. falciparum*; *Toxoplasma spp.*, including *T. gondii* (for example SAG2, SAG3, Tg34); *Entamoeba spp.*, including *E. histolytica*; *Babesia spp.*, including *B. microti*; *Trypanosoma spp.*, including *T. cruzi*; *Giardia spp.*, including *G. lamblia*; *Leshmania spp.*, including *L. major*; *Pneumocystis spp.*, including *P. carinii*; *Trichomonas spp.*, including *T. vaginalis*; *Schisostoma spp.*, including *S. mansoni*, or derived from yeast such as *Candida spp.*, including *C. albicans*; *Cryptococcus spp.*, including *C. neoformans*.

Other preferred specific antigens for *M. tuberculosis* are for example Rv2557, Rv2558, RPFs: Rv0837c, Rv1884c, Rv2389c, Rv2450, Rv1009, aceA (Rv0467), PstS1, (Rv0932), SodA (Rv3846), Rv2031c 16kDal., Tb Ra12, Tb H9, Tb Ra35, Tb38-1, Erd 14, DPV, MTI, MSL, mTTC2 and hTCC1 (WO 99/51748). Proteins for *M. tuberculosis* also include fusion proteins and variants thereof where at least two, preferably three polypeptides of *M. tuberculosis* are fused into a larger protein. Preferred fusions include Ra12-TbH9-Ra35, Erd14-DPV-MTI, DPV-MTI-MSL, Erd14-DPV-MTI-MSL-mTCC2, Erd14-DPV-MTI-MSL, DPV-MTI-MSL-mTCC2, TbH9-DPV-MTI (WO 99/51748).

Most preferred antigens for Chlamydia include for example the High Molecular Weight Protein (HWMP) (WO 99/17741), ORF3 (EP 366 412), and putative membrane

proteins (Pmps). Other Chlamydia antigens of the vaccine formulation can be selected from the group described in WO 99/28475.

Preferred bacterial vaccines comprise antigens derived from *Streptococcus spp.*, including *S. pneumoniae* (PsaA, PspA, streptolysin, choline-binding proteins) and the protein antigen Pneumolysin (Biochem Biophys Acta, 1989, 67, 1007; Rubins et al., Microbial Pathogenesis, 25, 337-342), and mutant detoxified derivatives thereof (WO 90/06951; WO 99/03884). Other preferred bacterial vaccines comprise antigens derived from *Haemophilus spp.*, including *H. influenzae* type B (for example PRP and conjugates thereof), non typeable *H. influenzae*, for example OMP26, high molecular weight adhesins, P5, P6, protein D and lipoprotein D, and fimbrial and fimbrial derived peptides (US 5,843,464) or multiple copy variants or fusion proteins thereof.

The antigens that may be used in the present invention may further comprise antigens derived from parasites that cause Malaria. For example, preferred antigens from *Plasmodia falciparum* include RTS,S and TRAP. RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P.falciparum* linked via four amino acids of the preS2 portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. Its full structure is disclosed in the International Patent Application No.

PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S. TRAP antigens are described in the International Patent Application No. PCT/GB89/00895, published under WO 90/01496. A preferred embodiment of the present invention is a Malaria vaccine wherein the antigenic preparation comprises a combination of the RTS, S and TRAP antigens. Other plasmodia antigens that are likely candidates to be components of a multistage Malaria vaccine are *P. falciparum* MSP1, AMA1, MSP3, EBA, GLURP, RAP1, RAP2, Sequestrin, PfEMP1, Pf332, LSA1, LSA3, STARP, SALSA, PfEXP1, Pfs25, Pfs28, PFS27/25, Pfs16, Pfs48/45, Pfs230 and their analogues in *Plasmodium spp.*

The invention contemplates the use of an anti-tumour antigen and be useful for the immunotherapeutic treatment of cancers. For example, tumour rejection antigens such as those for prostate, breast, colorectal, lung, pancreatic, renal or melanoma cancers.

Exemplary antigens include MAGE 1 , 3 and MAGE 4 or other MAGE antigens such as disclosed in WO99/40188, PRAME, BAGE, Lage (also known as NY Eos 1) SAGE and HAGE (WO 99/53061) or GAGE (Robbins and Kawakami, 1996, Current Opinions in Immunology 8, pps 628-636; Van den Eynde et al., International Journal of Clinical & Laboratory Research (submitted 1997); Correale et al. (1997), Journal of the National Cancer Institute 89, p293. Indeed these antigens are expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma.

5 MAGE antigens for use in the present invention may be expressed as a fusion protein with an expression enhancer or an Immunological fusion partner. In particular, the Mage protein may be fused to Protein D from *Heamophilus influenzae* B. In particular, the fusion partner may comprise the first 1/3 of Protein D. Such constructs are disclosed in Wo99/40188. Other examples of fusion proteins that may contain cancer specific epitopes include *bcr / abl* fusion proteins.

10 In a preferred embodiment prostate antigens are utilised, such as Prostate specific antigen (PSA), PAP, PSCA (PNAS 95(4) 1735 –1740 1998), PSMA or antigen known as Prostase.

15 Prostase is a prostate-specific serine protease (trypsin-like), 254 amino acid-long, with a conserved serine protease catalytic triad H-D-S and a amino-terminal pre-propeptide sequence, indicating a potential secretory function (P. Nelson, Lu Gan, C. 20 Ferguson, P. Moss, R. Gelinas, L. Hood & K. Wand, "Molecular cloning and characterisation of prostase, an androgen-regulated serine protease with prostate restricted expression, *In* Proc. Natl. Acad. Sci. USA (1999) 96, 3114-3119). A putative glycosylation site has been described. The predicted structure is very similar to other known serine proteases, showing that the mature polypeptide folds into a single domain. 25 The mature protein is 224 amino acids-long, with one A2 epitope shown to be naturally processed.

30 Prostase nucleotide sequence and deduced polypeptide sequence and homologs are disclosed in Ferguson, et al. (Proc. Natl. Acad. Sci. USA 1999, 96, 3114-3119) and in International Patent Applications No. WO 98/12302 (and also the corresponding granted patent US 5,955,306), WO 98/20117 (and also the corresponding granted patents US 5,840,871 and US 5,786,148) (prostate-specific kallikrein) and WO 00/04149 (P703P).

The present invention provides antigens comprising prostase protein fusions based on prostase protein and fragments and homologues thereof ("derivatives"). Such derivatives are suitable for use in therapeutic vaccine formulations which are suitable for the treatment of a prostate tumours. Typically the fragment will contain at least 20, 5 preferably 50, more preferably 100 contiguous amino acids as disclosed in the above referenced patent and patent applications.

A further preferred prostate antigen is known as P501S, sequence ID no 113 of WO98/37814. Immunogenic fragments and portions encoded by the gene thereof comprising at least 20, preferably 50, more preferably 100 contiguous amino acids as 10 disclosed in the above referenced patent application, are contemplated. A particular fragment is PS108 (WO 98/50567).

Other prostate specific antigens are known from Wo98/37418, and WO/004149. Another is STEAP PNAS 96 14523 14528 7 –12 1999.

Other tumour associated antigens useful in the context of the present invention 15 include: Plu –1 J Biol. Chem 274 (22) 15633 –15645, 1999, HASH –1, HasH-2, Cripto (Salomon et al Bioessays 199, 21 61 –70,US patent 5654140) Criptin US patent 5 981 215, ., Additionally, antigens particularly relevant for vaccines in the therapy of cancer also comprise tyrosinase and survivin.

The present invention is also useful in combination with breast cancer antigens such 20 as Muc-1, Muc-2, EpCAM, her 2/ Neu, mammaglobin (US patent 5668267) or those disclosed in WO/00 52165, WO99/33869, WO99/19479, WO 98/45328. Her 2 neu antigens are disclosed inter alia, in US patent 5,801,005. Preferably the Her 2 neu 25 comprises the entire extracellular domain (comprising approximately amino acid 1 –645) or fragments thereof and at least an immunogenic portion of or the entire intracellular domain approximately the C terminal 580 amino acids . In particular, the intracellular portion should comprise the phosphorylation domain or fragments thereof. Such constructs are disclosed in WO00/44899. A particularly preferred construct is known as ECD PD a second is known as ECD PD. (See WO/00/44899.)

The her 2 neu as used herein can be derived from rat, mouse or human.

30 The vaccine may also contain antigens associated with tumour-support mechanisms (e.g. angiogenesis, tumour invasion) for example tie 2, VEGF.

Vaccines of the present invention may also be used for the prophylaxis or therapy of

chronic disorders in addition to allergy, cancer or infectious diseases. Such chronic disorders are diseases such as asthma, atherosclerosis, and Alzheimers and other auto-immune disorders. Vaccines for use as a contraceptive may also be considered.

5 Antigens relevant for the prophylaxis and the therapy of patients susceptible to or suffering from Alzheimer neurodegenerative disease are, in particular, the N terminal 39 –43 amino acid fragment (AB₃₉ the amyloid precursor protein and smaller fragments. This antigen is disclosed in the International Patent Application No. WO 99/27944 – (Athena Neurosciences).

10 Potential self-antigens that could be included as vaccines for auto-immune disorders or as a contraceptive vaccine include: cytokines, hormones, growth factors or extracellular proteins, more preferably a 4-helical cytokine, most preferably IL13.

Cytokines include, for example, IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL11, IL12, IL13, IL14, IL15, IL16, IL17, IL18, IL20, IL21, TNF, TGF, GMCSF, MCSF and OSM. 4-helical cytokines include IL2, IL3, IL4, IL5, IL13, GMCSF and MCSF.

15 Hormones include, for example, luteinising hormone (LH), follicle stimulating hormone (FSH), chorionic gonadotropin (CG), VGF, GHrelin, agouti, agouti related protein and neuropeptide Y. Growth factors include, for example, VEGF.

20 The vaccines of the present invention are particularly suited for the immunotherapeutic treatment of diseases, such as chronic conditions and cancers, but also for the therapy of persistent infections. Accordingly the vaccines of the present invention are particularly suitable for the immunotherapy of infectious diseases, such as Tuberculosis (TB), HIV infections such as AIDS and Hepatitis B (HepB) virus infections.

25 The nucleotide sequence may be RNA or DNA including genomic DNA, synthetic DNA or cDNA. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. In order to obtain expression of the antigenic peptide within mammalian cells, it is necessary for the nucleotide sequence encoding the antigenic peptide to be presented in an appropriate vector system. By “appropriate vector” as used herein is meant any vector that will enable the antigenic peptide to be expressed within a mammal in sufficient quantities to evoke an immune response.

30 For example, the vector selected may comprise a plasmid, promoter and polyadenylation/ transcriptional termination sequence arranged in the correct order to obtain expression of the antigenic peptides. The construction of vectors which include

these components and optionally other components such as enhancers, restriction enzyme sites and selection genes, such as antibiotic resistance genes, is well known to persons skilled in the art and is explained in detail in Maniatis *et al* "Molecular Cloning: A Laboratory Manual", Cold Spring Harbour Laboratory, Cold Spring Harbour Press, Vols 1-3, 2nd Edition, 1989.

5 As it is preferred to prevent the plasmids replicating within the mammalian host and integrating within the chromosomal DNA of the animal, the plasmid will preferably be produced without an origin of replication that is functional in eukaryotic cells.

10 The methods and compositions according to the present invention can be used in relation to prophylactic or treatment procedures of all mammals including, for example, domestic animals, laboratory animals, farm animals, captive wild animals and, most preferably, humans.

15 The present inventors have demonstrated that the vaccination methods of the present invention are capable of enhancing both Th1 and Th2 cytokine profiles. However, there is a preferential shift towards a TH1 type of response.

20 A preferential inducer of a TH1 type of immune response enables a cell mediated response to be generated. High levels of Th1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of Th2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

25 It is important to remember that the distinction of Th1 and Th2-type immune response is not absolute. In reality an individual will support an immune response which is described as being predominantly Th1 or predominantly Th2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (Mosmann, T.R. and Coffman, R.L. (1989)

30 TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173). Traditionally, Th1-type responses are associated with the production of the INF- γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of Th1-type immune responses are not produced by T-cells, such as IL-12. In contrast, Th2-type responses are associated with the secretion of IL-4, IL-5, IL-6, IL-10.

The immunogen component comprising a vector which comprises the nucleotide sequence encoding an antigenic peptide can be administered in a variety of manners. It is

possible for the vector to be administered in a naked form (that is as naked nucleotide sequence not in association with liposomal formulations, with viral vectors or transfection facilitating proteins) suspended in an appropriate medium, for example a buffered saline solution such as PBS and then injected intramuscularly, subcutaneously, intraperitoneally or intravenously, although some earlier data suggests that intramuscular or subcutaneous injection is preferable (Brohm *et al* Vaccine 16 No. 9/10 pp 949-954 (1998), the disclosure of which is included herein in its entirety by way of reference). It is additionally possible for the vectors to be encapsulated by, for example, liposomes or within polylactide co-glycolide (PLG) particles (25) for administration via the oral, nasal or pulmonary routes in addition to the routes detailed above.

It is also possible, according to a preferred embodiment of the invention, for intradermal administration of the immunogen component, preferably via use of gene-gun (particularly particle bombardment) administration techniques. Such techniques may involve coating of the immunogen component on to gold beads which are then administered under high pressure into the epidermis, such as, for example, as described in Haynes *et al* J. Biotechnology 44: 37-42 (1996). In this context the antigen and the imidazo [4,5-c] quinolin – 4 – amine derivative can be co-formulated onto the same bead, or alternatively the antigen and the imidazo [4,5-c] quinolin – 4 – amine derivative can be separate. For example, the imidazo [4,5-c] quinolin – 4 – amine derivative may be administered topically at the site of administration of the DNA beads, as a cream, before or after administration of the DNA beads.

The vectors which comprise the nucleotide sequences encoding antigenic peptides are administered in such amount as will be prophylactically or therapeutically effective. The quantity to be administered, is generally in the range of one picogram to 1 milligram, preferably 1 picogram to 10 micrograms for particle-mediated delivery, and 10 micrograms to 1 milligram for other routes of nucleotide per dose. The exact quantity may vary considerably depending on the species and weight of the mammal being immunised, the route of administration, the potency and dose of the 1H-imidazo-[4,5-c]quinolin derivative, the nature of the disease state being treated or protected against, the capacity of the subject's immune system to produce an immune response and the degree of protection or therapeutic efficacy desired. Based upon these variables, a medical or veterinary practitioner will readily be able to determine the appropriate dosage level.

The imidazo [4,5-c] quinolin – 4 – amine derivative adjuvant component specified herein can similarly be administered via a variety of different administration routes, such as for example, via the oral, nasal, pulmonary, intramuscular, subcutaneous, intradermal or topical routes. Preferably, the component is administered via the intradermal or topical routes. This administration may take place between about 14 days prior to and about 14 days post administration of the nucleotide sequence, preferably between about 1 day prior to and about 3 days post administration of the nucleotide sequence. Most preferred is when the adjuvant component is administered substantially simultaneously with the administration of the nucleotide sequence. By "substantially simultaneous" what is meant is that administration of the adjuvant component is preferably at the same time as administration of the nucleotide sequence, or if not, at least within a few hours either side of nucleotide sequence administration. In the most preferred treatment protocol, the adjuvant component will be administered substantially simultaneously to administration of the nucleotide sequence. Obviously, this protocol can be varied as necessary, in accordance with the type of variables referred to above.

Once again, depending upon such variables, the dose of administration of the derivative will also vary, but may, for example, range between about 0.1mg per kg to about 100mg per kg, where "per kg" refers to the body weight of the mammal concerned. This administration of the 1H-imidazo[4,5-c]quinolin-4-amine derivative would preferably be repeated with each subsequent or booster administration of the nucleotide sequence. Most preferably, the administration dose will be between about 1mg per kg to about 50mg per kg.

While it is possible for the adjuvant component to comprise only 1H-imidazo[4,5-c]quinolin-4-amine derivatives to be administered in the raw chemical state, it is preferable for administration to be in the form of a pharmaceutical formulation. That is, the adjuvant component will preferably comprise the 1H-imidazo[4,5-c]quinolin-4-amine combined with one or more pharmaceutically or veterinarily acceptable carriers, and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with other ingredients within the formulation, and not deleterious to the recipient thereof. The nature of the formulations will naturally vary according to the intended administration route, and may be prepared by methods well known in the pharmaceutical art. All methods include the step of bringing into association a 1H-

imidazo[4,5-c]quinolin-4-amine derivative with an appropriate carrier or carriers. In general, the formulations are prepared by uniformly and intimately bringing into association the derivative with liquid carriers or finely divided solid carriers, or both, and then, if necessary, shaping the product into the desired formulation. Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a pre-determined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

10 A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

15 The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient.

20 Formulations for injection via, for example, the intramuscular, intraperitoneal, or subcutaneous administration routes include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Formulations suitable for pulmonary administration via the buccal or nasal cavity are presented such that particles containing the active ingredient, desirably having a diameter 25 in the range of 0.5 to 7 microns, are delivered into the bronchial tree of the recipient. Possibilities for such formulations are that they are in the form of finely comminuted powders which may conveniently be presented either in a piercable capsule, suitably of,

for example, gelatine, for use in an inhalation device, or alternatively, as a self-propelling formulation comprising active ingredient, a suitable liquid propellant and optionally, other ingredients such as surfactant and/or a solid diluent. Self-propelling formulations may also be employed wherein the active ingredient is dispensed in the form of droplets of a solution or suspension. Such self-propelling formulations are analogous to those known in the art and may be prepared by established procedures. They are suitably provided with either a manually-operable or automatically functioning valve having the desired spray characteristics; advantageously the valve is of a metered type delivering a fixed volume, for example, 50 to 100 μ L, upon each operation thereof.

5 In a further possibility, the adjuvant component may be in the form of a solution for use in an atomiser or nebuliser whereby an accelerated airstream or ultrasonic agitation is employed to produce a fine droplet mist for inhalation.

10 Formulations suitable for intranasal administration generally include presentations similar to those described above for pulmonary administration, although it is preferred for 15 such formulations to have a particle diameter in the range of about 10 to about 200 microns, to enable retention within the nasal cavity. This may be achieved by, as appropriate, use of a powder of a suitable particle size, or choice of an appropriate valve. Other suitable formulations include coarse powders having a particle diameter in the range of about 20 to about 500 microns, for administration by rapid inhalation through 20 the nasal passage from a container held close up to the nose, and nasal drops comprising about 0.2 to 5% w/w of the active ingredient in aqueous or oily solutions. In one embodiment of the invention, it is possible for the vector which comprises the nucleotide sequence encoding the antigenic peptide to be administered within the same formulation as the 1H-imidazo[4,5-c]quinolin-4-amine derivative. Hence in this embodiment, the 25 immunogenic and the adjuvant component are found within the same formulation.

20 In a preferred embodiment the adjuvant component is prepared in a form suitable for gene-gun administration, and is administered via that route substantially simultaneous to administration of the nucleotide sequence. For preparation of formulations suitable for use in this manner, it may be necessary for the 1H-imidazo[4,5-c]quinolin-4-amine derivative to be lyophilised and adhered onto, for example, gold beads which are suited 30 for gene-gun administration.

In an alternative embodiment, the adjuvant component may be administered as a dry powder, via high pressure gas propulsion. This will preferably be substantially simultaneous to administration of the nucleotide sequence.

Even if not formulated together, it may be appropriate for the adjuvant component to be administered at or about the same administration site as the nucleotide sequence.

5 Other details of pharmaceutical preparations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania (1985), the disclosure of which is included herein in its entirety, by way of reference.

The present invention will now be described further, with reference to the following 10 non-limiting examples:

Examples

1. Imiquimod increases the magnitude of the cytotoxic T cell response to a nucleic acid 15 vaccine.

Construction of plasmids and DNA preparation

PVAC1.ova.cyt

20 The plasmids used are based upon pVAC1, obtained from Michelle Young, GlaxoWellcome, UK, a modification of the mammalian expression vector, pCI, (Promega), where the multiple cloning site, from EcoRI to Bst ZI, has been replaced by the EMCV IRES sequence flanked 5' by unique Nhe I, Rsr II and Xho I and 3' by unique Pac I, Asc I and Not I restriction enzyme sites.

25 Ovalbumin (OVA) expressing plasmid pVAC1.ova.cyt was constructed by ligating the OVA sequence, into the expression vector pVAC1.

30 Plasmid DNA was propagated in *E. coli*, and prepared using plasmid purification kits (QIAGEN Ltd, Crawley, UK), and stored at -20°C at approximately 1 mg plasmid DNA/ml in 10 mM Tris/EDTA buffer.

A plasmid expressing the Gag and Nef antigens (ie.WRG7077Gag/Nef) was constructed based on WRG7077. The original WRG7077 plasmid was constructed by replacing the 5 beta-lactamase gene containing Eam1105I – PstI fragment of pUC19 (available from Amersham Pharmacia Biotech UK Ltd., Amersham Place, Little Chalfont, Bucks, HP7 9NA) with an EcoRI fragment of pUC4K (Amersham-Pharmacia) containing the Kanamycin resistance gene, following blunt ending of both fragments using T4 DNA polymerase. The human Cytomegalovirus IE1 promoter/enhancer, intron A, was derived 10 from plasmid JW4303 obtained from Dr Harriet Robinson, University of Massachussets, and inserted into the Sal1 site of pUC19 as a XhoI –Sal1 fragment, incorporating the bovine growth hormone polyadenylation signal. The Gag-Nef fusion was generated by PCR stitching of a truncated Nef with 195bp deleted from the 5' end of the gene removing the first 65 amino acids, derived from HIV-1 strain 248A (Genbank Acc. No. 15 L15518, a kind gift from G. Thompson), and p17p24 (Gag) from the plasmid pHXBΔPr (Maschera *et al.*, 1995) containing HIV-1 clade B strain HXB2 (Genbank Acc. No. K03455). The resulting Gag-Nef fusion was subsequently Ligated into WRG7077 as a NotI –BamHI fragment. Plasmid DNA and cartridge preparation was as described in example 1.

20

Preparations of cartridges for DNA immunisation

Preparation of cartridges for the Accell gene transfer device was as previously described (Eisenbraun et al DNA and Cell Biology, 1993 Vol 12 No 9 pp 791-797; Pertner et al).

25 Briefly, plasmid DNA was coated onto 2 µm gold particles (DeGussa Corp., South Plainfield, N.J., USA) and loaded into Tefzel tubing, which was subsequently cut into 1.27 cm lengths to serve as cartridges and stored desiccated at 4°C until use. In a typical vaccination, each cartridge contained 0.5 mg of gold beads coated with either ~0.05 µg pVAC1.ova.cyt (supplemented with the empty pVAC1 vector to provide a total of 0.5 µg 30 DNA/cartridge) or with 0.5µg WRG7077.gag/nef.

Example 2, Ovalbumin vaccinations

To examine whether the timing of imiquimod administration could affect the numbers of antigen specific cytokine producing cells in splenocytes pVAC1.ova.cyt (prepared according to example 1) was administered by particle mediated gene transfer (0.05 µg/cartridge) into the skin of mice. Plasmid was delivered to the shaved target site of abdominal skin of C57Bl/6 mice (purchased from Charles River United Kingdom Ltd, Margate, UK) from two cartridges using the Accell gene transfer device at 500 lb/in² (McCabe WO 95/19799).)

10

If given, immediately following vaccination imiquimod (prepared as a suspension in vehicle which comprised 0.3%(w/v) methylcellulose and 0.1% (v/v) Tween in sterile water) was administered by a single subcutaneous injection (0.05ml/10g body weight formulated to provide a dose of 30mg/kg) at the immunisation site. Plasmid and imiquimod controls were empty vector (pVAC1) and vehicle, respectively.

All mice received pVAC1.ova.cyt or empty vehicle pVAC1 at day 0 and week 4. One group of mice received imiquimod at day 0 only (Im prime), a second group of mice received imiquimod at week 4 only (Im boost) and another group received imiquimod at day 0 and week 4 (im pr + boost).

At day 12 splenocytes were collected and IFN-γ and IL-2 producing cells were measured by ELISPOT. The results are shown in FIG 1 and FIG 2.

25

Example 3, HIV vaccinations

Cartridges were prepared using the WRG7077Gag/Nef plasmid were prepared as described in example 1, and immunisations and cytokine producing cell responses were as described in example 2 using either WRG7077 vehicle alone or WRG7077.gag/nef.

30

Imiquimod was administered subcutaneously at a dose of 30 mg/kg. Spleens were collected for analysis 6 and 11 days after the boost immunisation.

The results are shown in FIG 3.

Claims

1. A method of vaccinating an individual comprising the steps of:

(c) vaccinating the individual with a first vaccine composition on one or more

5 occasions, characterised in that said vaccine comprises an antigen but does not comprise an imidazo [4,5-c] quinolin – 4 – amine derivative, and

(d) after waiting an appropriate length of time; vaccinating the same individual one or more times with a second vaccine, characterised in that the second vaccine composition comprises the same antigen as the first vaccine, the second vaccine being administered

10 with an imidazo [4,5-c] quinolin – 4 – amine derivative.

2. The method of vaccinating an individual as claimed in claim 1 further comprising a repeat of step (a) after step (b).

3. The method of vaccinating an individual as claimed in claim 1 wherein the second vaccine composition comprising the imidazo [4,5-c] quinolin – 4 – amine derivative is the final vaccine dose administered.

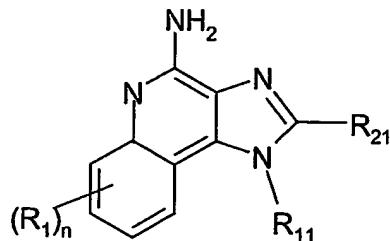
15 4. Use of an imidazo [4,5-c] quinolin – 4 – amine derivative and an antigen in the manufacture of a booster dose of a vaccine medicament for administration to an individual, characterised in that the individual previously received a priming dose of the vaccine medicament comprising the same antigen but which did not comprise an imidazo [4,5-c] quinolin – 4 – amine derivative.

20 5. A vaccine administration device comprising an antigen and an imidazo [4,5-c] quinolin – 4 – amine derivative, the device being packaged together with an instruction leaflet advising that the administration device is used to administer the vaccine composition only to individuals that had previously received a vaccine comprising the same antigen but which did not comprise an imidazo [4,5-c] quinolin – 4 – amine derivative.

25 6. A kit comprising a first vaccine composition and a second vaccine composition, wherein the first vaccine composition and the second composition contain the same antigen characterised in that the second vaccine composition comprises an imidazo [4,5-c] quinolin – 4 – amine derivative.

30 7. A method as claimed in claim 1 wherein the antigen and imidazo [4,5-c] quinolin – 4 – amine derivative are administered substantially simultaneously.

8. A method according to claim 1 or 2 wherein the 1H-imidazo[4,5-c]quinolin-4-amine derivative is a compound defined by one of formulae I-VI:

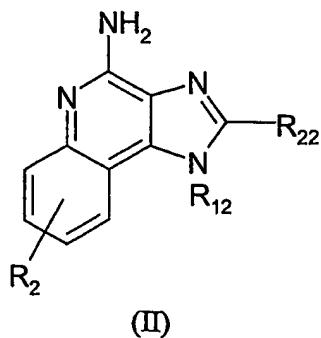


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(I)

wherein

R₁₁ is selected from the group consisting of straight or branched chain alkyl, hydroxyalkyl, acyloxyalkyl, benzyl, (phenyl)ethyl and phenyl, said benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two 10 moieties independently selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms and halogen, with the proviso that if said benzene ring is substituted by two of said moieties, then said moieties together contain no more than 6 carbon atoms; R₂₁ is selected from the group consisting of hydrogen, alkyl of one to about eight carbon atoms, benzyl, (phenyl)ethyl and phenyl, the 15 benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms and halogen, with the proviso that when the benzene ring is substituted by two of said moieties, then the moieties together contain no more than 6 carbon atoms; and each R₁ is independently 20 selected from the group consisting of hydrogen, alkoxy of one to about four carbon atoms, halogen and alkyl of one to about four carbon atoms, and n is an integer from 0 to 2, with the proviso that if n is 2, then said R₁₁ groups together contain no more than 6 carbon atoms;



wherein

R₁₂ is selected from the group consisting of straight chain or branched chain alkenyl

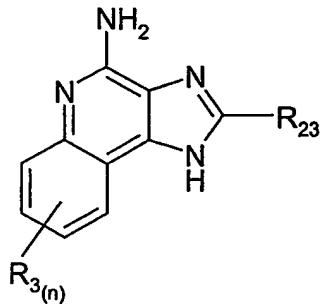
5 containing 2 to about 10 carbon atoms and substituted straight chain or branched chain alkenyl containing 2 to about 10 carbon atoms, wherein the substituent is selected from the group consisting of straight chain or branched chain alkyl containing 1 to about 4 carbon atoms and

cycloalkyl containing 3 to about 6 carbon atoms; and cycloalkyl containing 3 to about 6

10 carbon atoms substituted by straight chain or branched chain alkyl containing 1 to about 4 carbon atoms; and R₂₂ is selected from the group consisting of hydrogen, straight chain or branched chain alkyl containing one to about eight carbon atoms, benzyl, (phenyl)ethyl and phenyl, the benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group

15 consisting of straight chain or branched chain alkyl containing one to about four carbon atoms, straight chain or branched chain alkoxy containing one to about four carbon atoms, and halogen, with the proviso that when the benzene ring is substituted by two such moieties, then the moieties together contain no more than 6 carbon atoms; and each R₂ is independently selected from the group consisting of straight chain or branched chain

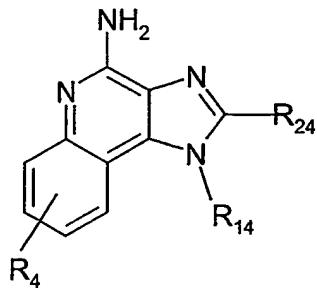
20 alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms, and n is an integer from zero to 2, with the proviso that if n is 2, then said R₂ groups together contain no more than 6 carbon atoms;



(III)

wherein

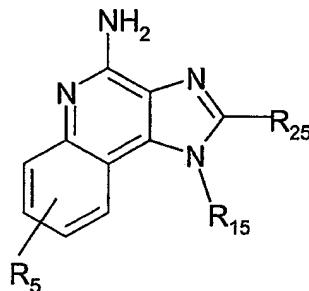
5 R₂₃ is selected from the group consisting of hydrogen, straight chain or branched chain alkyl of one to about eight carbon atoms, benzyl, (phenyl)ethyl and phenyl, the benzyl, (phenyl)ethyl or phenyl substituent being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of straight chain or branched chain alkyl of one to about four carbon atoms, straight chain or branched chain
10 alkoxy of one to about four carbon atoms, and halogen, with the proviso that when the benzene ring is substituted by two such moieties, then the moieties together- contain no more than 6 carbon atoms; and each R₅ is independently selected from the group consisting of straight chain or branched chain alkoxy of one to about four-carbon atoms, halogen, and 30 straight chain or branched chain alkyl of one to about four carbon atoms,
15 and n is an integer from zero to 2, with the proviso that if n is 2, then said R₃ groups together contain no more than 6 carbon atoms;



(IV)

20 wherein

R_{14} is $-\text{CHR}_A\text{R}_B$ wherein R_B is hydrogen or a carbon-carbon bond, with the proviso that when R_B is hydrogen R_A is alkoxy of one to about four carbon atoms, hydroxyalkoxy of one to about four carbon atoms, 1-alkynyl of two to about ten carbon atoms, tetrahydropyran, alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about four carbon atoms, 2-, 3-, or 4-pyridyl, and with the further proviso that when R_B is a carbon-carbon bond R_B and R_A together form a tetrahydrofuryl group optionally substituted with one or more substituents independently selected from the group consisting of hydroxy and hydroxyalkyl of one to about four carbon atoms; R_{24} is selected from the group consisting of hydrogen, alkyl of one to about four carbon atoms, phenyl, and substituted phenyl wherein the substituent is selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms, and halogen; and R_4 is selected from the group consisting of hydrogen, straight chain or branched chain alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms;



(V)

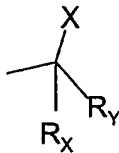
wherein

R_{15} is selected from the group consisting of: hydrogen; straight chain or branched chain alkyl containing one to about ten carbon atoms and substituted straight chain or branched chain alkyl containing one to about ten carbon atoms, wherein the substituent is selected from the group consisting of cycloalkyl containing three to about six carbon atoms and cycloalkyl containing three to about six carbon atoms substituted by straight chain or branched chain alkyl containing one to about four carbon atoms; straight chain or branched chain alkenyl containing two to about ten carbon atoms and substituted straight

chain or branched chain alkenyl containing two to about ten carbon atoms, wherein the substituent is selected from the group consisting of cycloalkyl containing three to about six carbon atoms and cycloalkyl containing three to about six carbon atoms substituted by straight chain or branched chain alkyl containing one to about four carbon atoms;

5 hydroxyalkyl of one to about six carbon atoms; alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about six carbon atoms; acyloxyalkyl wherein the acyloxy moiety is alkanoyloxy of two to about four carbon atoms or benzoxyloxy, and the alkyl moiety contains one to about six carbon atoms; benzyl; (phenyl)ethyl; and phenyl; said benzyl, (phenyl)ethyl or phenyl substituent
 10 being optionally substituted on the benzene ring by one or two moieties independently selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms, and halogen, with the proviso that when said benzene ring is substituted by two of said moieties, then the moieties together contain no more than six carbon atoms;

15 R_{25} is



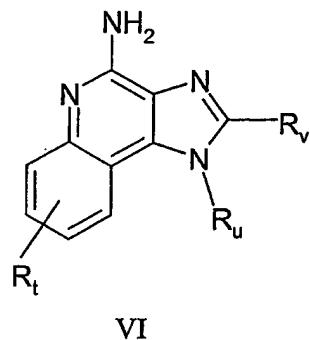
wherein

R_X and R_Y are independently selected from the group consisting of hydrogen, alkyl of one

20 to about four carbon atoms, phenyl, and substituted phenyl wherein the substituent is selected from the group consisting of alkyl of one to about four carbon atoms, alkoxy of one to about four carbon atoms, and halogen; X is selected from the group consisting of alkoxy containing one to about four carbon atoms, alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about four carbon atoms, haloalkyl of one to about four carbon atoms, alkylamido
 25 wherein the alkyl group contains one to about four carbon atoms, amino, substituted amino wherein the substituent is alkyl or hydroxyalkyl of one to about four carbon atoms, azido, alkylthio of one to about four carbon atoms; and R_5 is selected from the group

consisting of hydrogen, straight chain or branched chain alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms;

5



10

Wherein

R_t is selected from the group consisting of hydrogen, straight chain or branched chain alkoxy containing one to about four carbon atoms, halogen, and straight chain or branched chain alkyl containing one to about four carbon atoms;

15 R_u is 2-methylpropyl or 2-hydroxy-2-methylpropyl; and

R_v is hydrogen, alkyl of one to about six carbon atoms, or alkoxyalkyl wherein the alkoxy moiety contains one to about four carbon atoms and the alkyl moiety contains one to about four carbon atoms.

20 or a pharmaceutically acceptable salt of any of the foregoing.

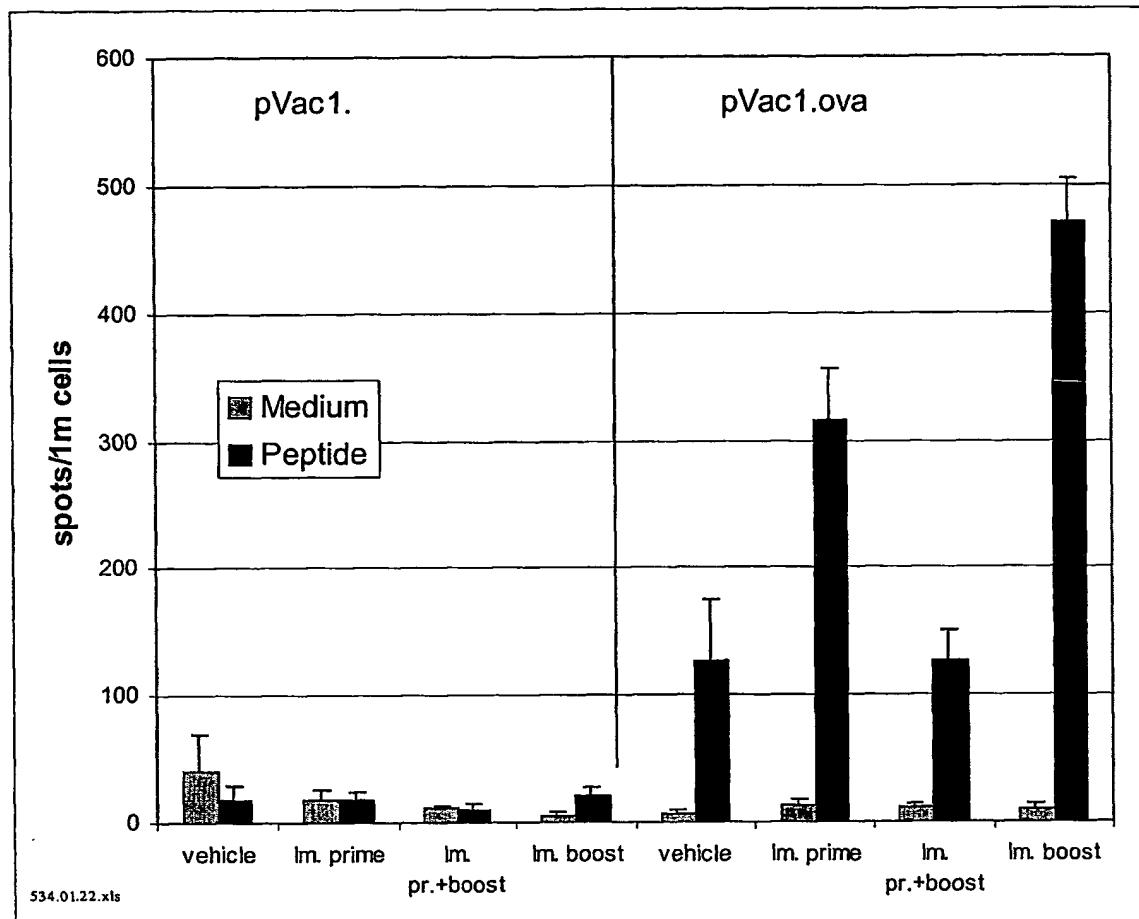
FIG 1. Anti-OVA IFN- γ producing splenocytes.

FIG 2, Anti-OVA IL-2 producing splenocytes

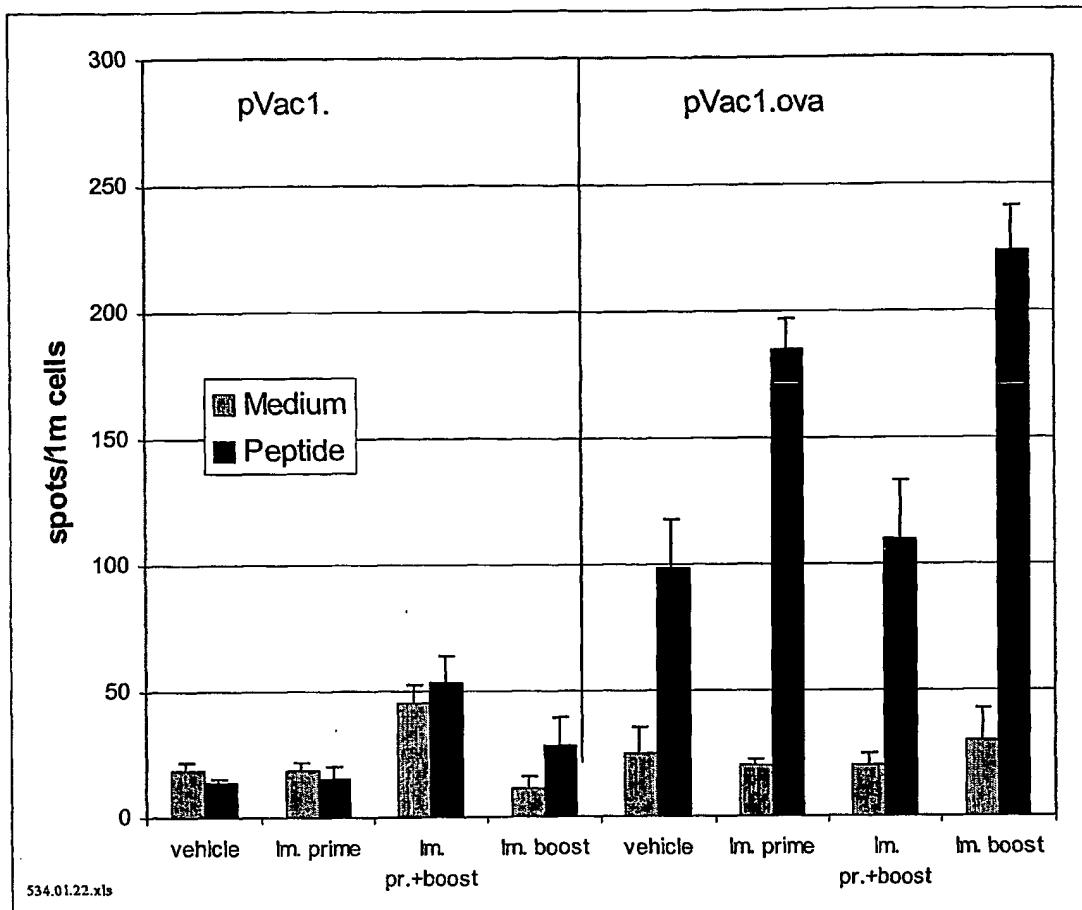
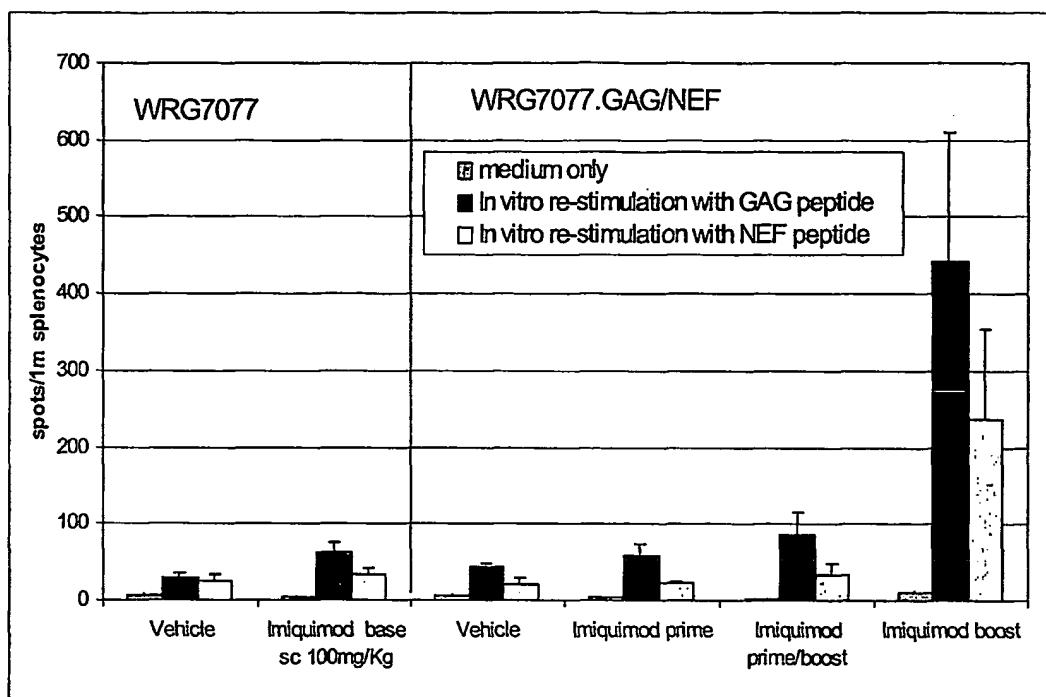


FIG 3, HIV specific IFN- γ producing splenocytes

INTERNATIONAL SEARCH REPORT

PCT/EP 03/02878

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 083 505 A (BERNSTEIN DAVID I ET AL) 4 July 2000 (2000-07-04) the whole document ---	1-8
X	BERNSTEIN D I ET AL: "ADJUVANT EFFECTS OF IMIQUIMOD ON A HERPES SIMPLEX VIRUS TYPE 2 GLYCOPROTEIN VACCINE IN GUINEA PIGS" JOURNAL OF INFECTIOUS DISEASES, CHICAGO, IL, US, vol. 167, no. 3, March 1993 (1993-03), pages 731-735, XP001037924 ISSN: 0022-1899 abstract; figure 1; table 1 page 732, column 1 --- -/-	1-8

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

30 July 2003

Date of mailing of the international search report

12/08/2003

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INTERNATIONAL SEARCH REPORT

PCT/EP 03/02878

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HARRISON CHRISTOPHER J ET AL: "Reduction of recurrent HSV disease using imiquimod alone or combined with a glycoprotein vaccine." VACCINE, vol. 19, no. 13-14, 2001, pages 1820-1826, XP002249629 ISSN: 0264-410X abstract; figures 1,2 page 1821 -----	1-8
X	VASILAKOS J P ET AL: "ADJUVANT ACTIVITIES OF IMMUNE RESPONSE MODIFIER R-848: COMPARISON WITH CPG ODN" CELLULAR IMMUNOLOGY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 204, no. 1, 25 August 2000 (2000-08-25), pages 64-74, XP001037913 ISSN: 0008-8749 abstract; figures 1-6 page 66 -page 68 -----	1-8
A	WO 00 47719 A (3M INNOVATIVE PROPERTIES CO) 17 August 2000 (2000-08-17) the whole document -----	1-8
P,X	WO 02 24225 A (GLAXO GROUP LTD ;TOPLEY PETER (GB); THOMSEN LINDY LOUISE (GB); TIT) 28 March 2002 (2002-03-28) cited in the application the whole document -----	1-8

INTERNATIONAL SEARCH REPORT**PCT/EP 03/02878****Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-4, 7 and 8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

Continuation of Box I.1

Rule 39.1(iv) PCT – Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

PCT/EP 03/02878

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
US 6083505	A	04-07-2000		AT 142110 T AU 674313 B2 AU 4048093 A DE 69304521 D1 DE 69304521 T2 DK 636031 T3 EP 0636031 A1 ES 2092306 T3 HK 1007962 A1 HU 69993 A2 HU 9500752 A3 IL 105325 A JP 7505883 T KR 263804 B1 MX 9302199 A1 NO 943920 A NZ 252020 A NZ 280098 A WO 9320847 A1 ZA 9302627 A		15-09-1996 19-12-1996 18-11-1993 10-10-1996 20-02-1997 24-02-1997 01-02-1995 16-11-1996 30-04-1999 28-09-1995 28-11-1995 14-11-1996 29-06-1995 16-08-2000 31-08-1994 14-10-1994 21-12-1995 26-06-1998 28-10-1993 14-10-1994
WO 0047719	A	17-08-2000		US 6558951 B1 AU 2504300 A CA 2362377 A1 EP 1153122 A2 JP 2002536013 T NO 20013875 A WO 0047719 A2		06-05-2003 29-08-2000 17-08-2000 14-11-2001 29-10-2002 08-10-2001 17-08-2000
WO 0224225	A	28-03-2002		AU 8790801 A CA 2422863 A1 EP 1318835 A1 WO 0224225 A1 NO 20031274 A		02-04-2002 28-03-2002 18-06-2003 28-03-2002 19-05-2003